

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently Amended) A purified α -isomaltosylglucosaccharide-forming enzyme, which forms a saccharide, having a glucose polymerization degree of at least three and having both the α -1,6 glucosidic linkage as a linkage at the non-reducing end and the α -1,4 glucosidic linkage other than the linkage at the non-reducing end, by catalyzing the α -glucosyl-transfer from a saccharide, having a glucose polymerization degree of at least two and having the α -glucosidic linkage as a linkage at the non-reducing end, in a substrate solution without substantially increasing the reducing power of the substrate solution; and having an amino acid sequence of ~~SEQ ID NO:1, 11 or 18~~ and molecular weight of ~~about 94,000 \pm 20,000 or about 140,000 \pm 20,000 daltons on SDS-PAGE.~~ Said enzyme solution having the following physicochemical properties:

- a. Molecular weight about 140,000 \pm 20,000 Daltons on SDS-PAGE;
- b. Isoelectric point (pI) about 5.2 \pm 0.5 or 7.3 \pm 0.5 on isoelectrophoresis using ampholine;
- c. Optimum temperature

- (i). About 40 to 50°C when incubated at a pH of 6.0 for 60 minutes;
 - (ii). About 45 to 55°C when incubated at a pH of 6.0 for 60 minutes in the presence of 1 mM Ca²⁺;
- d. Optimum pH about 6.0 to 6.5 when incubated at 35°C for 60 minutes;
- e. Thermal stability
 - (i). Stable up to a temperature of about 45°C when incubated at a pH of 6.0 for 60 minutes;
 - (ii). Stable up to a temperature of about 50°C when incubated at a pH of 6.0 for 60 minutes in the presence of 1 mM Ca²⁺;
and
- f. pH stability stable at pHs of about 4.5 to 10.0 when incubated at 4°C for 24 hours.

2. (Cancelled)

3. (Currently amended) The purified α -isomaltosylglucosaccharide-forming enzyme of claim 1 or 48, wherein said saccharide, having a glucose polymerization degree of at least two and having the α -1, 4 glucosidic linkage as a linkage at the non-reducing end, is one or more

members selected from the group consisting of
maltooligosaccharides, maltodextrins, amyloextrins, amyloses,
amylopectins, soluble starches, liquefied starches, and
glycogens.

4. (Cancelled)

5. (Cancelled)

6. (Cancelled)

7. (Cancelled)

8. (Currently Amended) A process for producing
the purified α -isomaltosylglucosaccharide-forming enzyme of
~~any one of claims~~ claim 1, 4 or 4448, which comprises:

- a. culturing in a nutrient culture
medium a microorganism capable of
producing said enzyme;
- b. and collecting said enzyme from the
resulting culture.

9. (Original) The process of claim 8, wherein
said microorganism is of the genus *Bacillus* or *Arthrobacter*.

10. (Original) The process of claim 9, wherein
said microorganism of the genus *Bacillus* is one selected from
the group consisting of *Bacillus globisporus* C9, FERM BP-7143;
Bacillus globisporus C11, FERM BP-7144; *Bacillus globisporus*
N75, FERM BP-7591; and mutants thereof.

11. (Original) The process of claim 9, wherein said microorganism of the genus *Arthrobacter* is one selected from the group consisting of *Arthrobacter globiformis* A19, FERM BP-7590; and mutant thereof.

12. (Currently Amended) A method of α -glucosyl-transferring reaction, which comprises a step of contacting the purified α -isomaltosylglucosaccharide-forming enzyme of ~~any one of claims 1, 4 or 44~~claim 1 or 48 with a solution comprising a saccharide having a glucose polymerization degree of at least two and having the α -1,4 glucosidic linkage as a linkage at the non-reducing end.

13. (Currently Amended) The method of claim 12, wherein a saccharide-transferred product is formed by the α -glucosyl-transferring reaction in the presence of one or more acceptors selected from the group consisting of D-glucose, D-xylose, L-xylose, D-galactose, D-fructose, D-mannose, D-arabinose, D-fucose, D-psicose, L-sorbose, methyl- β - glucopyranoside, methyl- β α -glucopyranoside, N-acetylglucosamine, trehalose, isomaltose, isomaltotriose, cellobiose, gentibiose, glycerol, maltitol, lactose, sucrose, and L-ascorbic acid.

14. (Currently Amended) A method for forming α -isomaltosyl- glucosaccharide, which comprises a step of contacting the purified α -isomaltosylgluco-saccharide-forming enzyme of claims 1, ~~4~~ or ~~44-48~~ with a solution, comprising a saccharide having a glucose polymerization degree of at least two and having the α -1,4 glucosidic linkage as a linkage at the non-reducing end, to effect α -glucosyl-transferring reaction.

15. (Original) The method of claim 14, wherein said saccharide is one selected from the group consisting of maltooligosaccharides, maltodextrins, amyloextrins, amyloses, amylopectins, soluble starches, liquefied starches, and glycogens.

Claims 16 - 42. (Deleted)

43. (Currently Amended) The purified α -isomaltosylglucosaccharide-forming enzyme of claim ~~41~~, ~~wherein~~ thewhich is derived from a microorganism of the genus *Bacillus* ~~is one~~-selected from the group consisting of *Bacillus globisporus* C9, FERM BP-7143; *Bacillus globisporus* C11, FERM BP-7144; and *Bacillus globisporus* N75, FERM BP-7591; and mutants thereof.

Claims 44-45. (Cancelled)

46. (Currently Amended) A biologically pure culture containing the α -isomaltosylglucosaccharide-forming enzyme of claims 1, 4, or ~~44~~48.

47. (New) The purified α -isomaltosylglucosaccharide-forming enzyme of claim 1, wherein said enzyme has a partial amino acid sequence of SEQ ID NO:1 or SEQ ID NO:11.

48. (New) A purified α -isomaltosylglucosaccharide-forming enzyme which forms a saccharide having a glucose polymerization degree of at least three and having both an α -1,6-glucosidic linkage as a linkage at the non-reducing end and an α -1,4-glucosidic linkage other than the linkage at the non-reducing end, by catalyzing α -glucosyl transfer from a saccharide having a glucose polymerization degree of at least two and having an α -1,4-glucosidic linkage as a linkage at the non-reducing end, in a substrate solution without substantially increasing the reducing power of the substrate solution;

Said enzyme having the following physicochemical properties:

- a. Molecular weight
- b. about 94,000 \pm 20,000 Daltons on SDS-PAGE;
- c. Isoelectric point (pI)

- d. about 4.3 ± 0.5 on isoelectrophoresis using ampholine;
- e. Optimum temperature
 - (i). About 60°C when incubated at a pH of 8.4 for 60 minutes;
 - (ii). About 65°C when incubated at a pH of 8.4 for 60 minutes in the presence of 1 mM Ca^{2+} ;
- f. Optimum pH about 8.4 when incubated at 35°C for 60 minutes;
- g. Thermal stability
 - (i). Stable up to a temperature of about 55°C when incubated at a pH of 8.0 for 60 minutes;
 - (ii). Stable up to a temperature of about 60°C when incubated at a pH of 8.0 for 60 minutes in the presence of 1 mM Ca^{2+} ;
and
- h. pH stability stable at pHs of about 5.0 to 9.0 when incubated at 4°C for 24 hours.

49. (New) The α -isomaltosylglucosaccharide-forming enzyme of claim 48, which is derived from a microorganism of the genus *Arthrobacter* selected from the group consisting of

Arthrobacter globiformis A19, FERM BP-7590, and mutants thereof.

50. (New) The α -isomaltosylglucosaccharide-forming enzyme of claim 48 wherein the enzyme has a partial amino acid sequence of SEQ ID NO:18.

51. (New) The α -isomaltosylglucosaccharide-forming enzyme of claim 1 or 48, which is substantially incapable of forming dextran, inhibited by EDTA, and stabilized and/or activated by Ca^{2+} and Mn^{2+} .